# DIVISION RATE IN CILIATE PROTOZOA AS INFLUENCED BY THYROID CONSTITUENTS.[1]

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## INTRODUCTION.

In the very numerous studies which have been made to ascertain the effect of thyroid tissues and extracts on growth and differentiation, the material employed, whether used as a food for large organisms or as a component of a medium in which to breed smaller forms, has been taken in a very large proportion of instances, if not always, from some mammal, *e. g.*, cow, horse, or sheep. This has been the case even though the animal under observation may have been a mammal, a bird, an amphibian, or a protozoan.

Assuming that the doctrine of evolution is a fairly probable hypothesis it is only a natural if not necessary corollary that each of the several organs involved, as well as the organism as a whole, has experienced its own successive changes, its own evolutionary modifications. Variations, "continuous" and "discontinuous," have occurred in internal as well as in external organs, and these variations have involved the physiological value of the organs concerned, as well as their anatomy; so that, of glandular tissues, for example, the composition of the output has undergone phylogenetic changes, so to speak, during the process of descent of one phylum from another. It is *a priori* improbable, of course, that the chemical composition, and consequent stimulating potency, of the thyroid secretion is the same throughout the entire vertebrate phylum.

Apparently the earliest experimentation along the line with which this paper deals was carried out by Nowikoff ('08), who found that one effect of putting sheep thyroid into the medium in which *Paramœcium* was living was to cause it to divide more rapidly than normally.

Recently, Shumway ('14) has published a paper in which he verifies Nowikoff's contentions. Both these investigators, however, employed mammalian thyroid; and, since our results agree

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with theirs, the question for mammalian thyroid, at least, seems fairly well settled.

Nowikoff's work suggested to us the query whether or not his results could be taken as widely significant.[1] Our purpose has been, therefore, to add to the known facts along this line by ascertaining the influence of glands taken from each of the five main subdivisions of vertebrated animals, so far as they or substances derived from them, affect certain protozoa; and, using division-rate as an index, to thus get a line on the comparative physiology of this gland.

## Materials and Methods.

Perfectly fresh thyroid glands were taken from the fresh-water sucker (*Catostomus teres*), the frog (*Rana pipiens*), the turtle (*Cistudo carolina*), the chick (*Gallus domesticus*), and the cat (*Felis domestica*), dissected as cleanly as possible from surrounding tissues, and then dried by moderate heat; each was then ground to powder in a mortar, and the material then kept in vials till used. In the instance of the mammalian gland, fatty tissue was present in such amount that this was dissolved away by repeated washing in ether to bring the gland to such condition that it could be finely pulverized. In supplying thyroid material to protozoa in this form, we endeavored to avoid any alteration in its character such as might result in the making of glycerin or alcoholic extracts. This seems a point which should be rather carefully guarded.

The forms employed for experimentation were *Stylonichia* and *Paramœcium*. To familiarize ourselves with a method of handling such organisms, and also for the purpose of securing individuals whose ancestry would be known to us, we first carried isolated "wild" forms through a considerable number of generations (in the case of *Stylonichia*, seventy-four); we employed depression slides, kept in a moist chamber, each slide carrying four drops of bacterial hay infusion made up in the manner of that used by Woodruff ('05) in much of his work. The "wild" specimens were taken from ordinary laboratory cultures, but the particular individuals used in any given experiment were taken from the pedigreed lines descended from a single parent. The

[1] Our work was completed before Shumway's article appeared.

protoplasm of the line treated with thyroid was identical with that of the control carried beside it.

The procedure in any experiment was this: two protozoa of common parentage were isolated, each in four drops of the same culture medium. To one of the slides was added a minute mass of pulverized gland, which would thus influence the protozoan either as a food, or as a factor in the environing medium so far as this acted as a solvent.[1] The actual amount of each pulverized gland thus used was small, and a like amount of each was determined as closely as possible by careful subdivision of a slightly larger mass on a clean paper surface. To weigh out the powder would give no more equal amounts, inasmuch as the glands are so invaded by vascular and connective tissue that any moiety taken might easily contain more or less of other than glandular material. A slight amount of fresh hay infusion was added to each slide each day, and the experiment continued six days or more. The results given in this account are limited to those obtained during the first six days only; to keep track of the offspring of even a single protozoan longer than this is extremely difficult, as many know. The effect of each different gland was tested by three trials.

Circumstances were such that it was not always convenient or possible to run experiments with all five different thyroids at one time, so a control was carried along beside the gland-fed individual in each case. This assured that the same conditions of every sort attended both experimental and control lines, no matter when the observations were made. If any circumstance favored or interfered with either, the same was true for the other.

## Experimental Findings.

The following tables show the exact results, so far as number of individuals resulting from division of the original one goes, this rate of cell division being the only index of thyroid effect at present ready for presentation. The ciliate used in the first series of experiments was *Stylonichia;* in the second and third series we used *Paramœcium.* While the evidence is too limited to permit any rigid conclusion of the kind, the data at hand seem

[1] Shumway states in his recent paper, *loc. cit.*, that more or less of the material thus offered *Paramœcium* is actually ingested and digested.

to indicate that *Paramœcium* is rather more susceptible to thyroid ingredients than is *Stylonichia*.

RESULTS FROM USE OF FISH THYROID.

| | First Experiment, No. of Individuals. | | Second Experiment, No. of Individuals. | | Third Experiment, No. of Individuals. | |
|---|---|---|---|---|---|---|
| | Control. | Thyroid-fed. | Control. | Thyroid-fed. | Control. | Thyroid-fed. |
| 1st day | 1 | 1 | 1 | 1 | 1 | 1 |
| 2d day | 4 | 2 | 1 | 2 | 1 | 1 |
| 3d day | 6 | 6 | 3 | 2 | 6 | 6 |
| 4th day | 15 | 15 | 5 | 7 | 14 | 16 |
| 5th day | 24 | 37 | 7 | 50 | 18 | 23 |
| 6th day | 43 | 49 | 15 | 90 | 52 | 73 |

RESULTS FROM USE OF AMPHIBIAN THYROID.

| | First Experiment, No. of Individuals. | | Second Experiment, No. of Individuals. | | Third Experiment, No. of Individuals. | |
|---|---|---|---|---|---|---|
| | Control. | Thyroid-fed. | Control. | Thyroid-fed. | Control. | Thyroid-fed. |
| 1st day | 1 | 1 | 1 | 1 | 1 | 1 |
| 2d day | 1 | 1 | 1 | 2 | 1 | 2 |
| 3d day | 6 | 5 | 2 | 4 | 8 | 18 |
| 4th day | 7 | 11 | 8 | 9 | 16 | 76 |
| 5th day | 7 | 12 | 10 | 30 | 25 | 127 |
| 6th day | 12 | 30 | 12 | 61 | 36 | 243 |

RESULTS FROM USE OF REPTILIAN THYROID.

| | First Experiment, No. of Individuals. | | Second Experiment, No. of Individuals. | | Third Experiment, No. of Individuals. | |
|---|---|---|---|---|---|---|
| | Control. | Thyroid-fed. | Control. | Thyroid-fed. | Control. | Thyroid-fed |
| 1st day | 1 | 1 | 1 | 1 | 1 | 1 |
| 2d day | 4 | 4 | 4 | 5 | 1 | 2 |
| 3d day | 8 | 10 | 8 | 23 | 3 | 7 |
| 4th day | 8 | 10 | 8 | 47 | 15 | 42 |
| 5th day | 8 | 21 | 14 | 148 | 23 | 80 |
| 6th day | 8 | 38 | 15 | 362 | 50 | 276 |

RESULTS FROM USE OF AVIAN THYROID.

| | First Experiment, No. of Individuals. | | Second Experiment, No. of Individuals. | | Third Experiment, No. of Individuals. | |
|---|---|---|---|---|---|---|
| | Control. | Thyroid-fed. | Control. | Thyroid-fed. | Control. | Thyroid-fed. |
| 1st day | 1 | 1 | 1 | 1 | 1 | 1 |
| 2d day | 1 | 4 | 1 | 2 | 2 | 1 |
| 3d day | 2 | 9 | 3 | 7 | 8 | 16 |
| 4th day | 2 | 10 | 4 | 45 | 23 | 32 |
| 5th day | 4 | 15 | 15 | 63 | 59 | 141 |
| 6th day | 5 | 24 | 35 | 243 | 91 | 399 |

RESULTS FROM USE OF MAMMALIAN THYROID.

| | First Experiment, No. of Individuals. | | Second Experiment, No. of Individuals. | | Third Experiment, No. of Individuals. | |
|---|---|---|---|---|---|---|
| | Control. | Thyroid-fed. | Control. | Thyroid-fed. | Control. | Thyroid-fed. |
| 1st day | 1 | 1 | 1 | 1 | 1 | 1 |
| 2d day | 13 | 15 | 4 | 8 | 2 | 2 |
| 3d day | 16 | 18 | 6 | 18 | 9 | 18 |
| 4th day | 18 | 20 | 10 | 48 | 34 | 59 |
| 5th day | 30 | 132 | 14 | 60 | 56 | 129 |
| 6th day | 53 | 306 | 29 | 253 | 90 | 487 |

For convenience in seeing at a glance the relation which held between the experimental lines and the controls during the use of any particular kind of thyroid, the data for the three experiments with each thyroid have been averaged, the controls for the same averaged, and the results placed in curve form.[1] These follow:

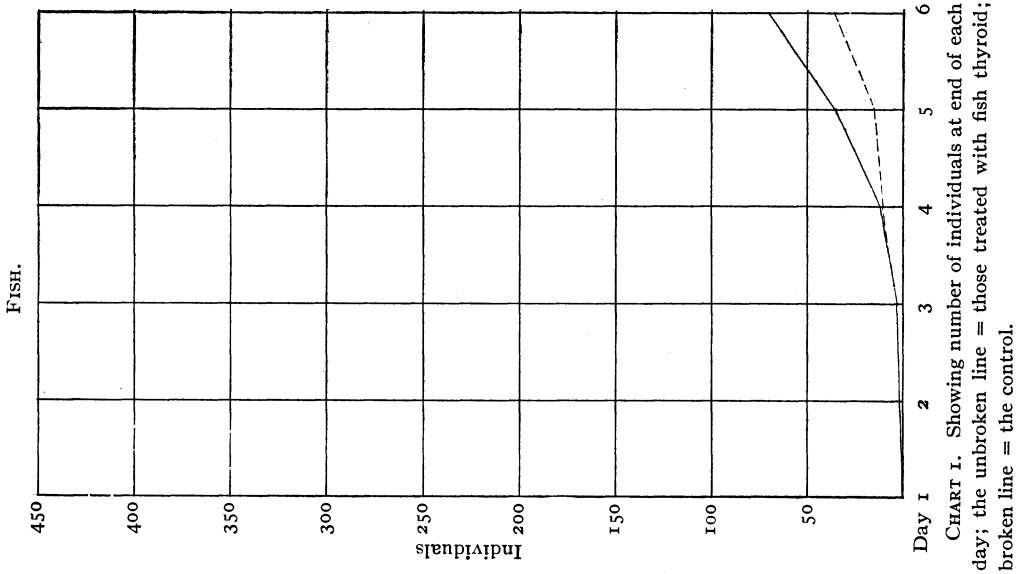


CHART 1. Showing number of individuals at end of each day; the unbroken line = those treated with fish thyroid; broken line = the control.

[1] An erroneous conclusion is rather easily drawn from these charts, for at first glance it appears that the potential of the gland increases by steady gradation from the fish up to the mammal. When figured as percentage increases of the experimental over the control lines, it is found that such is not the case. Data pertaining to this relation are now being collected.

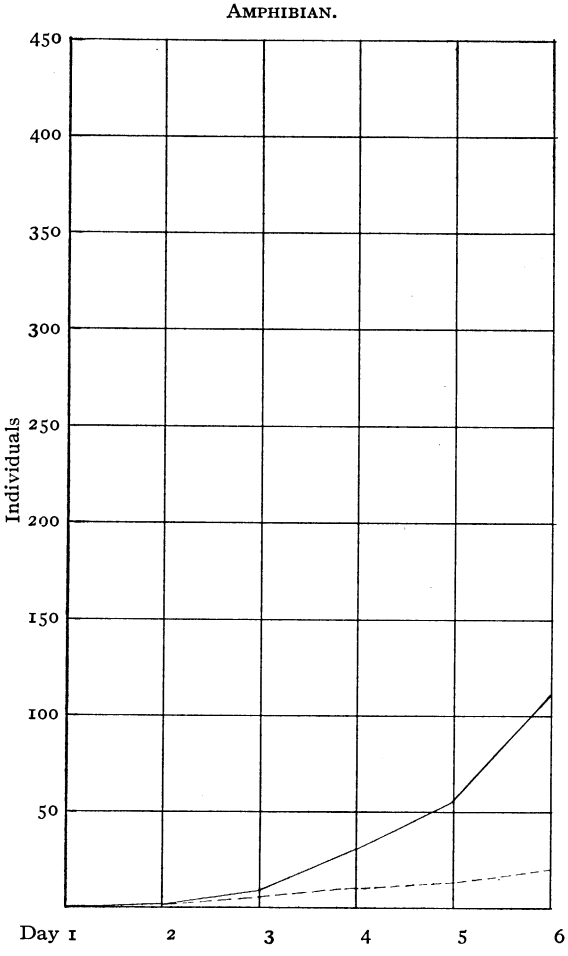


CHART 2. Plotted in same manner as Chart 1, but showing result when one of two sister individuals was given amphibian thyroid.

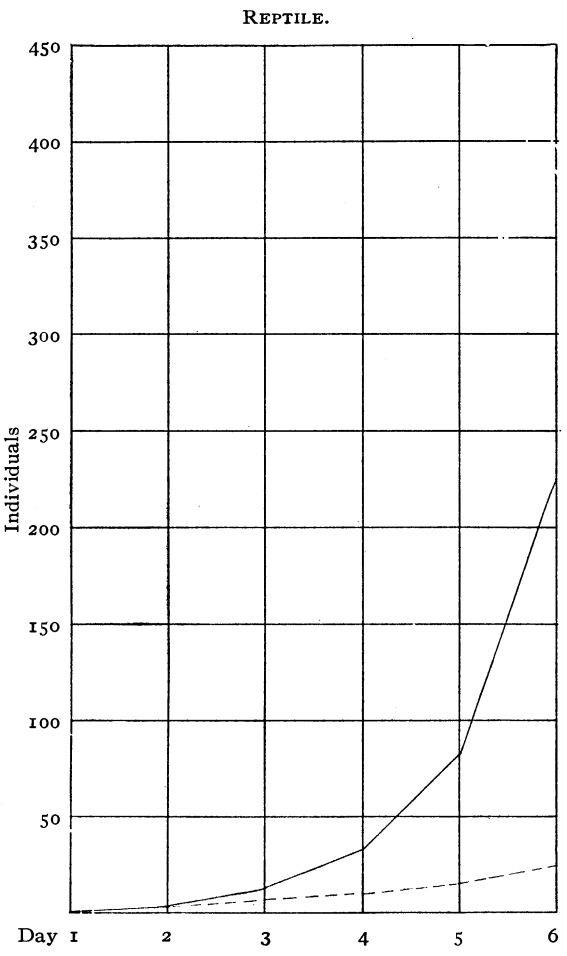


CHART 3. Plotted in same manner as Chart 1, but showing result secured with reptilian thyroid.

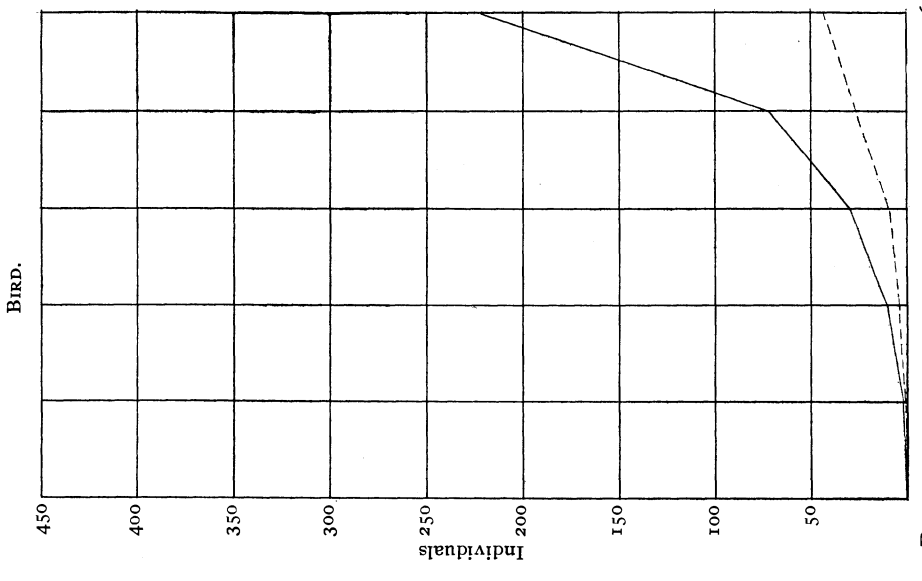


CHART 4. Plotted in same manner as Chart 1, but showing result secured with avian thyroid.

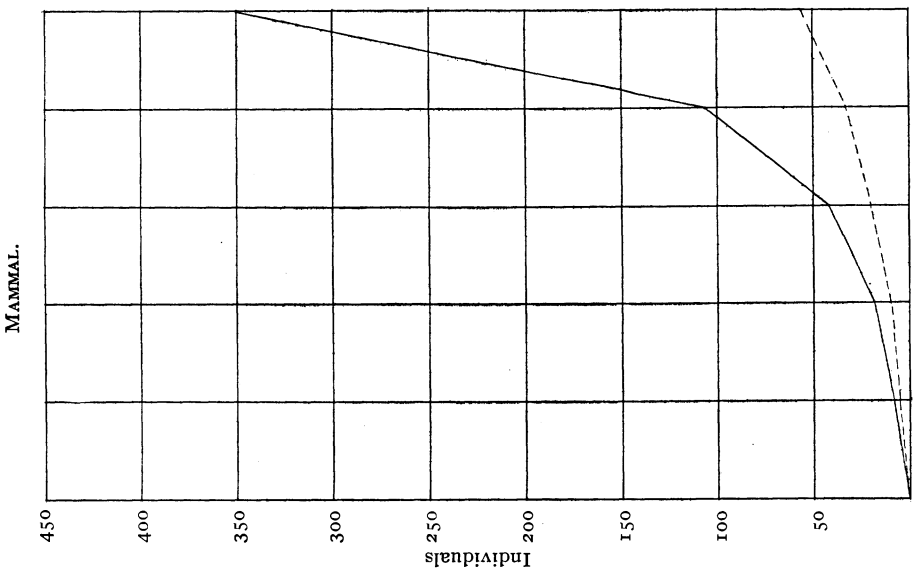


CHART 5. Plotted in same manner as Chart 1, but showing result secured with mammalian thyroid.

A curve plotted from the averages of all five of the different experimental lines and drawn beside a curve portraying the averages of the control lines for the same periods, each figured day by day for the six days, represents, in a manner, a generalized curve of the effect of vertebrate thyroid constituents on protozoa, as based on our data. This curve takes the following form:

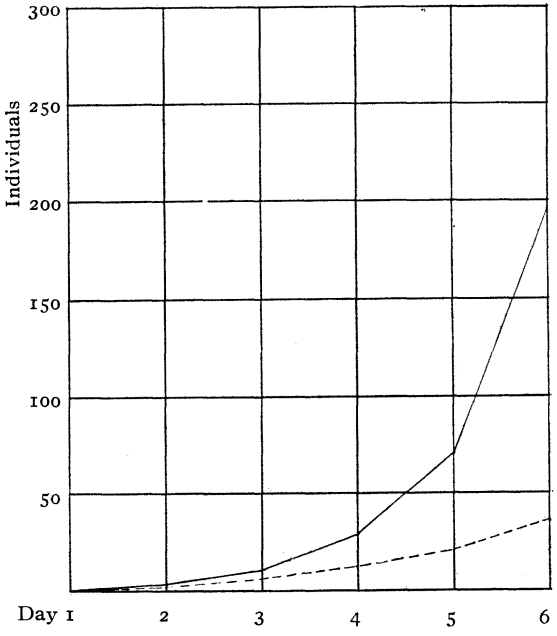


CHART 6. A curve plotted from the averages of Charts 1, 2, 3, 4 and 5, and thus representing a composite of results obtained from use of thyroids from each of the five main classes of vertebrates.

## DISCUSSION.

The number of papers which have hitherto been published along this line seems to be limited to those already mentioned; so that conclusions have to be drawn from a comparatively meager literature, and to gather largely around the investigator's

own experience. There is essential agreement between the findings of Nowikoff, Shumway, and ourselves as to the constant effect of thyroid ingredients in increasing the division rate of protozoa beyond the normal, at least so far as *Paramœcium* is concerned.

The work of others, notably Gudernatsch ('12, '13), on the feeding of amphibian embryos, seems to indicate that the effect there observed is mainly one of acceleration of differentiation of tissues in the growing organism; at least this is the interpretation given their findings. West ('14) has verified certain features of Gudernatsch's results. A similar betrayal of specialization in function would, of course, not be possible within the limits of a unicellular organism. It seems entirely probable, however, that intra-cellular modifications of the *Paramœcium* protoplasm does accompany its feeding upon and living in a medium which, among other things, brings it hurriedly to its most crucial experience, self-division. The fact that rapid fission of thyroid-fed *Paramœcia* is accompanied by their increased activity and transparency, and by smaller size,[1] indicates that very important internal modifications doubtless occur. Careful study of protozoa exposed to exigencies of this sort should be made.

If cell division in protozoa is to be compared with anything in the life history of metazoa, it should certainly be considered beside the early development of the metazoan egg. If the egg has already advanced to the proportions of an embryo or larva, and the precocious differentiation of tissues and organs in such is under consideration, the question arises: Is this differentiation at all explained in the same terms as is protozoan cell division, or does it involve the same basic factors? It seems to us that this query may very possibly be answered in the affirmative, for the reason that the sprouting out of legs from the tadpole and establishment of other organs characteristic of the adult, is surely not due to mere unusual division of labor among the young cells generally acting as little more than unit components of the infant tadpole body; but that these latter have been provoked (by thyroid ingredients?) to *abnormally rapid division*, probably

[1] Shumway mentions these alterations to occur in thryoid-fed *Paramœcia*, and we have found such to be practically always observable.

with accompanying abnormally small size, and that *entirely normal differentiation* has set in among cells which have been derived by the abnormally early (rapid) multiplication of their ancestors.

We would suggest, therefore, that there may be, at bottom, not any great difference between what shows itself in Gudernatsch's work as differentiation, and the result which shows itself as cell division in an animal where differentiation, so far as it exists, can assert itself only intra-cellularly, and thus in a very obscure manner.

It is certainly entirely unnecessary to dwell upon the obvious fact that the more nearly adult a metazoan animal is, the more difficult it becomes to even suggest parallelisms which may exist between it and unicellular organisms; so that, to discuss the numerous physiological effects which have been obtained from feeding thyroid tissues to various vertebrata, or from grafting and transplantation experiments, or to examine the studies of conditions in higher types provoked by pathological thyroid growth and disease, is quite beyond the scope, if not impossible in connection with the subject, of this paper.

## Summary.

The conclusion to which the foregoing experimental results point is that thyroid ingredients, no matter from what class of vertebrates the gland be taken, produce essentially the same result when given to ciliate protozoa (*Paramœcium* and *Stylonichia*) as a food or as a factor in the medium in which they live, viz., increased division rate.

The tissue which has hitherto been used in experimental work along this line has, we believe, always been taken from mammals. We think it safe to say that, no matter how far apart taxonomically, or how distantly related phylogenetically the "higher" and "lower" members of the vertebrate phylum may be, certain physiological qualities in the thyroid glands are constant and similar in all.

Sufficient difference exists between the potential of the thyroid secretion of one vertebrate class and that of another, so that, if studies of the normal value of this gland are being made, glands

from the same class, if not from the same genus and species of animal as the one under observation, may well be employed.

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